

FORMULATION AND CHARACTERIZATION OF EXTRACTIVE PREPARATIONS OF FRAGARIAE HERBA. EVALUATION OF THE DIURETIC EFFECT

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Abstract

The present study was aimed to obtain extractive products of *Fragariae herba*, harvested from the spontaneous flora of North-West Romania. References recommended *Fragaria* species for their diuretic effect without mentioning its dose and intensity. For a better display of this therapeutic effect, two types of products were developed – a fluid extract and a tincture – using a mix of diluted alcohol 70° (g/g), propylene glycol 10% and alcohol 90°. Once the extractive products were obtained, their physical-chemical characterization was determined, as well as their flavonoids concentration (active principles responsible for the therapeutic effect) using the method compendiale the flavonoid concentration was expressed in rutin. The diuretic effect was observed on a group of Wistar rats. Based on the experimental results it could be stated that the extracts had a diuretic effect that was similar to Furosemid, but without the Potassium losses - a favorable aspect for patients with cardiovascular diseases.

Key words: *Fragariae Herba*, diuretic effect, fluid extract

INTRODUCTION

Compendiums mention numerous medicinal herbs which can be used for their diuretic effect, as well as for ameliorating complications due to arterial hypertension (Tiță I., 2005).

The conducted study reviews pharmaceutical preparations that could possibly contain plant extracts with diuretic effects, based on plants collected from the spontaneous flora. Thus, the first part of the process consisted in preparing and determining the physical-chemical characterization of two extractive preparations with high flavonoid content, responsible for the diuretic effect. In order to evaluate their therapeutic effect, an *in vivo* method applied on Wistar rats was used.

MATERIAL AND METHOD

***Fragariae herba* extract. Preparation method**

A physical-chemical characterization of the obtained fluid extract was carried out, along with the standardization in flavonoids.

The following materials were used: dried and pulverized plants (according to FR X), diluted alcohol 70° (g/g) and propylene glycol 10%

Preparation method:

The extraction was carried out using repercolation by Squibb, known as radial percolation. The following stages were completed in order to prepare 1000 g of fluid extract: 3 percolators were used with the plant content distributed as:

I 500 g II 300 g III 200 g

500 g were macerated for 2 hours with diluted alcohol 70° (g/g) and 50 g of propylene glycol, then introduced in the percolator and filled with diluted alcohol 70° (g/g) until the first drops of extract appeared in the tube of the percolator. The percolator faucet was closed and the maceration was continued for 24 hours (Vicaş L.G., 2011).

After 24 hours, the faucet was opened, establishing a flow velocity of 7-8 drops/minute until 200 g of extract were gathered. The extract was introduced in a closed vessel and the rest of the extract was collected in a separate vessel. After collecting it, the second portion of the extract was immersed – 300 g with 100 g of diluted alcohol 70° (g/g) and 30 g of propylene glycol 10%; after approximately 2 hours, it was introduced in the percolator over the first portion, and the rest of the extract was used for the third portion.

The last portion of 200 g of plant extract was immersed with the rest of the second portion, 50 g respectively, 20 g of propylene glycol 10% and the rest of the extract was added, completing with diluted alcohol 70° (g/g). After 24 hours, 500 g of extract were collected and were mixed with the two above-mentioned portions, resulting in 1000 g of fluid extract.

After 5-7 days, the extract was filtered and was completed up to 1000 g with the remained extract from the third portion.

2. *Fragariae herba* tincture. Preparation method

The *Fragariae herba* tincture was obtained from the fresh, grinded plant and ethanol 90° (1:2), by maceration for 10 days at room temperature, stirring 3-4 times per day. The extractive solution was decanted, the residue was pressed and the resulted tincture was filtered (Popovici I. et al, 2011)

RESULTS AND DISSCUSIONS

Physical-chemical characterization – was carried out after the guidelines provided in FR X in the *Extracta* monograph; thus, the color, aspect, smell, taste, purity requirements (iron and heavy metals content), alcohol content and residue after evaporation were determined.

Tabel 1

Physical-chemical parameters of the fluid extract

Characteristics		Physical-chemical parameters
Aspect		Clear liquid
Color		Green-brown
Smell		Characteristic
Density		0.890
Purity requirements	-16 iron % g/g max.	0.100
	-17 heavy metals % g/g max.	0.100
Residue after evaporation % g/g min.		2.00
Alcohol content % g/g min.		48
Flavonoid content expressed in rutin % g/g		0.25 – 0.70

Standardization was expressed in flavonoid components, these substances presenting a better stability in time than the other active principles from the extract.

Their value was represented by the average of 5 samples collected from Băile Felix, Băile Tinca, Aleșd, Stâna de Vale.

For each sample, 2000 g of fluid extract were studied, their analysis being conducted after the Romanian Pharmacopeia 10th method provided in the *Cynarae folium* monograph.

The results were obtained using a calibration curve for a 0.01% rutin solution (Ph. Eur.8.0).

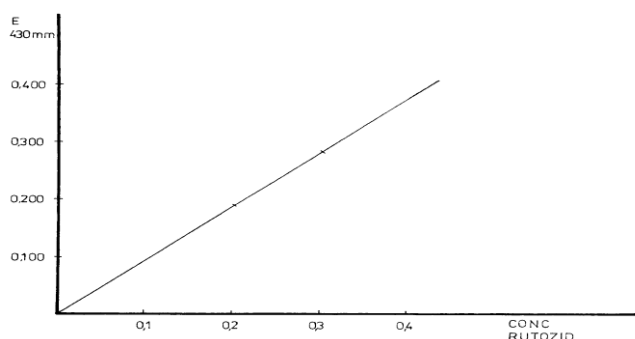


Fig. 1 Calibration curve for a 0.01% rutin solution

Tabel 2

Flavonoid content of the fluid extract

No. sample	Origin	Flavonoid content
1	Băile Felix	0.48
2	Băile Tinca	0.50
3	Sovata	0.72
4	Aleşd	0.25
5	Stâna de Vale	0.61

The flavonoid content of the fluid extract was comprised between 0.25 – 0.70 g%, expressed in rutin.

Physical-chemical characterization – the studied parameters were identical with the ones from the previous product, the methods used were provided by the *Tinctura* monograph in Romanian Pharmacopeia 10th.

Tabel 3

Physical-chemical characteristics of the *Fragariae herba* tincture

Characteristics		Physical-chemical parameters
Aspect		Clear liquid
Color		Green
Smell		Characteristic
Purity requirements	-16 iron % g/g max.	0.001
	-17 heavy metals % g/g max.	0.001
Residue after evaporation % g/g min.		0
Alcohol content % g/g min.		50-56
Flavonoid content expressed in rutin % g/g		0.25 – 0.50

Tabel 4

Flavonoid content of the tincture

No. sample	Origin	Flavonoid content
1	Băile Felix	0.48
2	Băile Tinca	0.50
3	Sovata	0.72
4	Aleşd	0.25
5	Stâna de Vale	0.61

The flavonoid content of the fluid extract was comprised between 0.10 – 0.30 g%, expressed in rutin.

The flavonoid substances were also found in the tincture, but in a lower percentage than the plant and the fluid extract; they were identified through chromatographic methods (Popovici M. et al, 2008).

3. Diuretic effect determination

Among the multiple properties of the plant, the diuretic effect is also mentioned.

Due to the great necessity of diuretic products, this pharmacological property was studied *in vivo*.

The fluid extract was dried and the residue was dissolved in water.

The diuretic effect was demonstrated on a group of 7 Wistar rats, weighing approximately 200 g.

In order to demonstrate the diuretic effect, a major diuretic – injectable furosemid solution, respectively - was used as a standard.

The substances were administered by oral gavage, as aqueous solutions, in quantities of 0.015 g fluid extract/100 g animal, which corresponds to 10 g fluid extract/70 kg for humans (N) and in doses 10 times larger than the normal one. The standard substance – furosemid, was administered as 20 mg/100g animal.

Tabel 5

No.	Animal weight (g)	Evaluation of the diuretic effect				Diuresis after 10 hours (ml)
		Sample	Active substance quantity/100 g animal (mg)	Solution volum (ml)	Water volum (ml)	
0	1	2	3	4	5	6
1	220	N	15	2,2	+2,8	3,50
2	210	N	15	2,1	+2,9	7,00
3	240	N	150	2,4	+2,6	5,00
4	260	N	150	2,6	+2,4	4,00
5	200	Furosemid	20	2,0	3,00	4,00
6	212	Water	-	-	5,00	1,20
7	200	Apă	-	-	5,00	1,00

Thus, in the normal dose, the fluid extract presented diuretic properties; the eliminated urine volume values averaged in comparison to the standard diuretic – furosemid, as illustrated in the table above (Cristea A.N., 2005). The results were also similar in the case of the 10 times larger doses.

Apart from the diuretic effect, the natriuresis and kaliuresis were determined as well, thus a pronounced sodium elimination was observed, comparable to the furosemid; however, the potassium elimination was maintained within normal limits in all cases.

Tabel 6

The natriuresis and kaliuresis determined							
Sam ple	1	2	3	4	5	6	7
Na ⁺	19	37	56	50	37	13	15
K ⁺	6	7	11	15	7	7	5

CONCLUSIONS

The undertaken study has confirmed that the plant classified in the diuretics category, thus it could be included in the composition of several pharmaceutical specialties with diuretic properties.

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